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TITLE: A Multidisciplinary Approach to Study the Role of the Gut Microbiome in Relapsing and Progressive MS

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14. ABSTRACT The project is both scientifically and financially on track. In the last year we have concentrated in recruiting the subjects to analyze during the third and final year of this project. Recruitment of RRMS and control subjects is close to target, while recruitment of PPMS subjects is proceeding at a slower pace, as anticipated. We plan to stop recruitment in February 2018, and proceed to process samples for sequencing and genotyping in order to complete the project in September of 2018. On the experimental side, a group of 24 germ-free mice was colonized with human microbiota and EAE was induced. We expect results of this experiment by November 2017. A second experiment to replicate our results will follow shortly.						
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1. INTRODUCTION

We hypothesize that specific human GI microbiota can alter the balance of inflammatory and regulatory immune cell populations thus leading to disease in genetically susceptible hosts. Furthermore, we hypothesize that gut microbiota from subjects with RMS and PPMS is fundamentally different and can elicit distinguishable effects when transferred into susceptible animal models of the disease.

2. KEYWORDS

Microbiome
Multiple sclerosis
Primary Progressive
Relapsing Remitting
Knockout mice
Bioinformatics
Immunology

3. ACCOMPLISHMENTS

3a. Major goals of the project: This project has two major goals or aims. In the SOW, each Aim was subdivided into Major Tasks and subtasks:

Aim#1: To compare the gut microbiome of subjects with RMS and PPMS.

Major Task 1: To seek and obtain HRPO approval –**COMPLETE**

Major Task 2: Identification and recruitment of research subjects -- **COMPLETE**

Subtask 1: Perform Chart reviews to identify eligible patients from MS clinic at UCSF and Mt Sinai. -- **COMPLETE**

Subtask 2: Clinical evaluation and invitation to participate in the study -- **COMPLETE**

Major Task 3: Sample collection and initial processing -- **ONGOING**

Subtask 1: Preparation of collection mailing kits -- **ONGOING**

Subtask 2: Bacterial DNA extraction from stool material -- **ONGOING**

Subtask 3: Genotyping and HLA characterization of host DNA. – **TO DO**

Milestone #1: Recruitment and processing samples from 150 RMS, 150 PPMS and 150 healthy controls.

Major Task 4: 16S ribosomal gene sequencing and initial bioinformatics analysis.

Subtask 1: sequencing of 16S ribosomal RNA gene in all DNA samples from MS patients and controls. – **TO DO**

Milestone #2: Sequencing of the MS microbiome.

Major Task 5: Data integration and advanced bioinformatics analysis.

Subtask 1: Integration of microbiome and genomic data – **TO DO**

Aim#2: To test the effect of human MS microbiota in spontaneous and induced experimental models.

Major Task 1: microbiota transfer into germ-free mice and EAE induction -- **ONGOING**

Subtask 1: re-derivation of Tob1/2D2 mice into a GF line -- **ONGOING**

Subtask 2: Transfer of live microbiota from select patients into germ-free mice, EAE induction and follow-up -- **ONGOING**

Major Task 2: Immuno-pathological characterization of experimental mice

Subtask 1: tissue dissection, harvesting and pathological analysis – **TO DO**

Subtask 2: Flow cytometry – **TO DO**

Subtask 3: Immunohistochemistry – **TO DO**

Subtask 4: Molecular characterization – **TO DO**

*Milestone #3: Co-authored manuscript – **TO DO***

3b. Accomplishments to date:

Subject recruitment is ongoing at both Mt. Sinai and UCSF. As of October 2017, Mt. Sinai recruited a total of 141 subjects (28 since our last quarterly report, 124 since our last annual report). Of these, 60 are RRMS, 11 PPMS and 70 healthy controls. Blood samples were collected for all subjects and clinical visits, stool samples, are either completed or in progress. Additional subjects are being screened with special emphasis on increasing our PPMS population

Similarly, UCSF recruited a total of 211 subjects (19 since our last quarterly report, 147 since our last annual report) subjects: 72 with RRMS, 67 with PPMS and 72 healthy controls. Blood and stool samples were collected from all subjects and all subjects completed their clinic visits. Case report questionnaires are either completed or in progress for all subjects.

In total, between both sites, we have recruited 352 subjects (132 RRMS, 83 PPMS and 142 controls).

Additional Accomplishments:

- HRPO approval has been obtained (Specific Aim 1. Major Task 1)
- Chart reviews are being conducted to identify eligible subjects for this study at UCSF and Mt Sinai (Specific Aim 1. Major Task 2. Subtask 1).
- Clinical evaluation and invitations to participate are being conducted (Specific Aim 1. Major Task 2. Subtask 2).
- Mailing kits have been designed, prepared and being distributed to eligible participants (Specific Aim 1. Major Task 3. Subtask 1).
- Bacterial DNA is being purified from stool samples. Quality control is being performed (Specific Aim 1. Major Task 3. Subtask 2)
- Tob1/2D2 mice are currently being derived germ-free (Specific Aim 2. Major Task 1. Subtask 1)

- Germ-free C57Bl/6 mice (7-8/group) have been colonized with whole microbiota from a RRMS, a PPMS and a healthy subject. After 5 weeks, EAE was induced. Experiment is ongoing at this time.

3c. Opportunities for training and professional development

Exchange of protocols and samples between experimental sites is ongoing. Caltech (Sub: Mazmanian) has received microbiota samples from UCSF (PI: Baranzini) to colonize germ-free mice. This experiment requires careful coordination between the Caltech, UCSF and Mt Sinai groups to transfer samples for analysis. Once the experiment is performed at Caltech, both UCSF and MT Sinai will receive specimens for analysis. Investigators at each of the 3 sites are in close communication. In addition, UCSF (PI: Baranzini) and ICSD (PI: Knight) investigators are permanent contact to process samples for bacterial DNA sequencing once recruitment has completed.

3d. Dissemination of results to communities of interest

Nothing to report. Since most of the activities so far have been concentrated on recruitment, there are no results to report yet. A related article from our groups (UCSF, Caltech, UCSD and MT Sinai) was published in PNAS and received significant media attention for its design, and impact (Cekanaviciute et al. PNAS 2017). This study was funded by the National MS Society and philanthropic contributions.

3e. Plans for accomplishing project goals during the next reporting period

The project is well underway. One identified challenge is that recruitment of PPMS subjects has been slower than anticipated. While this is not surprising (given the lower prevalence of this disease subtype), it poses a challenge the team needs to address. The Mt Sinai team is planning a “PPMS Science” day at a busy clinic in upstate New York in order to boost recruitment for this study. IRB approval has been already obtained.

4. IMPACT

4a. Impact on the development of the principal discipline(s) of the project

Nothing to report

4b. impact on other disciplines

Nothing to report

4c. impact on technology transfer

Nothing to report

4d. impact on society beyond science and technology

Nothing to report

5. CHANGES/PROBLEMS

5a. Changes in approach and reasons for change

Nothing to report

5b. Actual or anticipated problems or delays and actions or plans to resolve them

As anticipated, recruitment of the 150 primary progressive MS subjects is proving challenging. Strategies for identifying and targeting primary progressive MS patients for recruitment based on medical record review are being implemented (see 3e). We project that completing recruitment of the 150 PPMS subjects will cause a delay in the project. In any case, this delay will not result in additional expenses. One potential alternative is to analyze a smaller number of PPMS subjects. Based on our preliminary data, a sample size of ~100 (which we are confident we can reach in this following year) will be sufficient to identify major shifts in the gut microbiome. This strategy will be discussed among the project PIs and the DoD officials.

5c. Changes that had a significant impact on expenditures

Nothing to report

5d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

6. PRODUCTS

6a. Publications, conference papers, and presentations

A related article from our groups (UCSF, Caltech, UCSD and Mt Sinai) was published in PNAS and received significant media attention for its design, and potential impact in MS (Cekanaviciute et al. PNAS 2017). This study was funded by the National MS Society and philanthropic contributions.

6b. Website(s) or other Internet site(s)

Nothing to report

6c. Technologies or techniques

Nothing to report

6d. Inventions, patent applications, and/or licenses

Nothing to report

6e. Other Products (Reportable outcomes)

Aim 1 is in its recruiting phase. No reportable outcome at this time.
 Aim 2 is in progress. No reportable outcome at this time.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7a. Individuals working on the project

Personnel	Project PI	Role	Nearest person Month	Contribution
Sergio Baranzini	Baranzini	PI	1	Project overview
Bruce Cree	Cree	PI	1	Clinical PI
Sneha Singh	Cree	Coordinator	6	Clinical coordinator
Adam Santaniello	Cree	Database manager	1	Set up project database
Rob Knight	Knight	PI	1	Technical development PI
Amnon Amir	Knight	Post Doc	2	development of fast algorithms for OTU picking that will be used in the 16S analysis
Stefan Maximilian Janssen	Knight	Post Doc	2	development of methods to analyze exRNA sequences to assess interkingdom communication
Tomasz Piotr Kosciolek	Knight	Post Doc	3	development of methods to identify ncRNAs in the genomic and metagenomic data
Jon Sanders	Knight	Post Doc	3	development of genome annotation pipeline and fast matching for separating human reads from microbial that will be used in the shotgun metagenomics.
Daniel McDonald	Knight	Bioinformatic 1 s Programmer		computational studies of sequenced genomes, extracting and integrating biological information and data.
Jeffrey E Dereus	Knight	Programmer 2 Analyst		management of the software team, setup of project tracking and LIMS for this project.

7b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

A new R01 (NINDS) has been awarded to Sergio Baranzini (UCSF). This new award is not related to microbiome research (Title: Genetics of progression in MS).

7c. What other organizations were involved as partners?

Partner (sub-contract) 1

Organization Name: California Institute of Technology

Location of Organization: Pasadena, CA

Partner's contribution to the project (identify one or more): Germ-free mouse experiments

Partner (sub-contract) 2

Organization Name: Icahn School of Medicine at Mount Sinai

Location of Organization: New York, NY

Partner's contribution to the project (identify one or more): Subject recruitment and experimental work (immunohistochemistry of brain sections, immunophenotyping and RNAseq of selected mouse tissues)

8. SPECIAL REPORTING REQUIREMENTS:

This is a Collaborative award (3 Principal Investigators).

As approved by the Program official assigned to this project (Amie Bunker) the same report is being submitted under each PI's account.